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Invited Review Article

Cutting edge meticulous appraisal of equine piroplasmosis in India and in rest of the Globe

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Abstract

Equine piroplasmosis (EP) is an ixodid tick endured infection of equids (horses, mules, pony, donkeys and zebra) caused by two intra erytrocytic apicomplexan haemoprotozoan parasites i.e. *Babesia caballi* and *Theileria equi*. These parasites are also found in other animals like dog and camel pointing uncertainty about their host specificity. The introduction of carrier equids into a region where ixodid ticks exist in abundance can lead to an epizootic feast of the infection to naïve equid. This disease has distributed worldwide, besides *T. equi* has also been reported from various states of India. It is a serious threat to intercontinental maneuver of equid business affecting the health of adult animals as well as foal. EP can be controlled by drug therapy along with management interventions as till now no vaccine is available. Efficient and effective control steps should be taken to reduce the transmission of infection by the ticks. This review briefly emphasis on all features of the EP like ancient outline, parasitic life cycle, worldwide prevalence of the EP along with special emphases on Indian scenario, pathogenesis, clinical sign, haemato-biochemical observations, clinical pathology, diagnosis, treatment and prevention.

Introduction

Equine piroplasmosis (EP) a tick-borne haemoprotozoan disease of the equines caused by obligatory intra-erythrocytic protozoa *Babesia equi,* reclassified as *Theileria* (*T.*) *equi* (Mehlhorn and Schein 1998) and *Babesia* (*B.*) *caballi*. It may occur in an acute, sub-acute or chronic form. The disease was first reported in Sudan by Oliver (1907) cited in Abdoon (1984). Equine piroplasmosis is endemic in many tropical and subtropical areas of the world, including Europe, Asia, Africa, South and Central America. The disease has worldwide economic impact on the horse industry predominately in Asia, Europe, Africa and South American continent (Homer *et al* 2000).

Thoroughbred horses and other interrelated animals like donkeys, mules, pony and zebras etc. which come under the family Equidae (generally known as equids) are the potential risk prone animals to equine piroplasmosis. Around the globe the estimated equines population is to be tune of 114 million comprising 59 million horses, 44 million donkeys and 11 million mules (FAOSTAT, 2012) which may suffer for various bacterial, viral, fungal and parasitic diseases. Out of 114 million equids, more than 97% of the world's donkey and mule populations, and over 72% of horse population is in developing countries which is specifically kept for draft purpose. These animals are more prone to parasitic infections specifically haemoprotozoan disease. India has about 1.77 million equids, which are kept under different farming systems and are used for numerous purposes which constitute 5% of Asian equine population (Fazili and Kirmani, 2011). These equids also suffer for various infectious diseases including haemoprotozoan. Equine piroplasmosis (EP), also called as anthrax fever, equine malaria, equine biliary fever, equine babesiosis, horse tick fever or equine theileriosis is an important haemoprotozoan infection (Onyiche *et al.,* 2019). It is a notifiable disease of equids instigated by blood endured protozoan parasites i.e. *B caballi* and *B. equi.*

Equine piroplasmosis is responsible for noteworthy financial losses in the equids business due to financial damage caused management expenditure, abortion, loss of action and mortality (Onyiche *et al.,* 2019). First case of EP was reported in Sudan by Oliver (1907), infecting mainly the RBC and lymphocytes (*B. equi*). The trophozoites *B. equi* in Romanowsky stained blood smears appear as round, spindle or elliptical-shaped basophilic structures (Sumbria *et al.,* 2014). They are small erythrocytic stage piroplasms reaching only upto 1.5-2.5 µm with the merozoite stage appearing as two or four (Maltese cross) pyriform parasites, whereas on the other side the trophozoites of *B. caballi* appear as round, elliptical or oval basophilic structures with the erythrocytic stage reaching 3-6 µm. This organism commonly found in a single erythrocyte; transpire mainly in pairs forming an acute angle

(Edwards *et al.,* 2005). The Ixodid ticks of the genera *Boophilus, Hyalomma, Dermacentor, Rhipicephalus, Haemaphysalis* and *Amblyomma* are responsible for transmission of this apicomplexan haemoparasite. In India ticks of *Hyalomma* (*Hyalomma anatolicum*) species seem to be budding vectors for the transmission of *T. equi* in equids.

Life cycle

Theileria equi

It is a small form of haemoparasite which makes Maltese cross (4 parasite ar right angles) in RBC. Unlike *B. caballi*, *T. equi* has a stage of schizogony in the lymphocytes of equids host (Uilenberg, 2006). Within 5 days inside the salivary gland of infected blood fed tick vector, the haemoparasite develops to sporozoites stage (de Waal and van Heerden, 2004). This occurs after the infected adult tick has attached to a susceptible equid (Uilenberg, 2006). Infection occurs by injection of the infected saliva (having sporozoites) by ticks into the equids blood stream. In this case, the infected tick loses its Theilerial infection after transmission (Uilenberg, 2006). Within 12-14 days after infected ticks first attach to non-infected equid, sporozoites are released to invade lymphocyte, with in which it forms macro and micro schizonts. At the same time the merozoites invade RBC and occur either as two or four, in a Maltese cross formation and are seen as pyriform parasites (Vial and Gorenflot, 2006). Now after blood feeding in tick gametogony takes place i.e. ring stage are formed from merozoites which in turn form zygote by fusion of macrogamete and microgamete. Zygote gives rise to kinete formation, this result to sporoblast and in last sporozoites production (sporogony) occurs in salivary gland of tick.

Babesia caballi

The development of *B. caballi* occurs exclusively in the equid RBC (de Waal and van Heerden, 2004). When infected tick feed on equid blood, it will inject saliva along with parasitic sporozoites, these sporozoites get inside the RBCs and by schizogony they form pyriform merozoites and are often found as pairs with an acute angle, these merozoites then infect other young RBC and thus increase the parastemia. Now after blood feeding in tick's gut merozoits form ring stages which in turn form zygote by fusion of macrogamete and microgamete. Zygote gives rise to kinete formation, this result to sporoblast and in last sporozoites production occurs (de Waal and van Heerden, 2004).

Pathogenesis and Clinical Pathology

Disease entity in EP generally varies from host to host, it also varies according to host age, health status immunological status etc. In relation to pathogenesis generally the parasite (*B. caballi*) cause clumping of RBC so there is formation of micro-thrombi in small blood vessels, this result to venous stasis and vasculitis (de Waal *et al.,* 1987). Parasite also cause prolonged clotting times, thrombocytopenia and decrease in PCV (Allen *et al.,* 1975). In blood of equids the parasite causes lysis of RBC resulting in varying degrees of hemolytic anemia. Erythrophagocytic destruction of RBC from the circulation by macrophages enhances the chances of anemia. Moreover, cerebral forms are also observed in *B. caballi* infection. Out of the 2 parasite *T. equi* is highly pathogenic and can infect up to 80% of RBC (Mehlhorn and Schein, 1998). These parasites also change the biochemical structure of RBC membranes, which results in the change in the deformability of the RBC, and it result in reduction of microvascular blood flow. Other important factor of destruction of RBC is the accumulation of oxidative ions (Ambawat *et al.,* 1999). *Theileria equi* depend on the RBC for their energy supply; moreover, the increased uptake of phosphorus by the infected RBC may be responsible for the infected RBC's fragility and hypophosphataemia (de Waal *et al.,* 1988). This hypophosphataemia can lead to adenosine triphosphate (ATP) depletion, which predisposes to development of haemolysis. Concurrent infections, such as African horse sickness and verminosis can complicate equine theileriosis and thus may lead to disseminated intravascular coagulopathy (de Waal and van Heerden, 2004).

Infection can result in a variety of clinical signs. Clinical signs occur after an incubation period of 5-30 days after the bite of an infected tick during blood feeding (Phipps and Phipps, 1996). The disease may be per-acute, acute, sub-acute, or chronic (Rothschild and Knowles, 2007). In per-acute *T. equi* infection there is unforeseen onset of signs, which lead to collapse and sudden death of infected equid. EP may lead to haemolysis which in turn causes anaemia. *Babesia caballi* infected horses become less anaemic, but death from *B. caballi* mainly occurred due to multiple organ failure, which is due to systemic formation of micro-intravascular coagulation (Donnellan and Marais,

2009). Haemolytic anaemia result into icteric or pale mucous membranes, tachycardia, tachypnea, weakness, and pigmenturia (Zobba *et al.,* 2008).

In acute infection, initially there is high fever (104°F), weight loss, peripheral oedema, lethargy and anorexia. Petechial haemorrhages caused by thrombocytopenia are mainly observed on mucous membranes, including the nictitating membrane. Some equines show signs of gastrointestinal complication. Other sings include secondary development of cardiac arrhythmias, catarrhal enteritis, laminitis, pneumonia, pulmonary edema and central nervous system disease characterized by ataxia, seizures and myalgia (Diana *et al.,* 2007; Zobba *et al.,* 2008). Haemoglobin-induced pigment nephropathy and systemic responses to severe inflammation result in hypotension and acute renal failure (de Waal, 1992). In Sub acute cases there may be varying degree of anorexia, elevated or normal rectal temperature, weight loss, increased pulse and respiratory rates, colic, constipation followed by diarrhoea and sometimes haemoglobinuria. Pale-yellow to bright yellow mucous membranes also been seen. Strenuous exercise may predispose horses to the clinical manifestation of the disease (Hailat *et al.,* 1997).

Chronic infections lead to liver failure or disseminated intravascular coagulation (Donnellan and Marais 2009). Abortion or neonatal infection can occur in pregnant carrier mares (Allsopp *et al.,* 2007). Acute, severe signs develop in neonatal foals infected *in-utero* with *T. equi* (Georges *et al.,* 2011; Chhabra *et al.,* 2012). These foals can exhibit clinical signs at birth or can become ill at 2-3 days of age. Clinical signs in foals are nonspecific, such as weakness and decreased suckling ability, but with time the signs progress to resemble with those of an infected adult, including icterus, fever and anaemia. Chronic *T. equi* or *B. caballi* infection can result in lethargy, partial anorexia, weight loss, poor performance, mild anaemia and enlarged spleen which are non-specific signs. Splenic enlargement is caused by the increased rate of extra vascular haemolysis that occurs within the spleen (Allen *et al.,* 1975).

In relation to haemato-biochemical changes, the parasite causes anaemia, the level of packed cell volumes (PCV) may be as low as 20%, but seldom falls below 10 per cent. Thrombocytopenia is also commonly identified (Zobba *et al.,* 2008). Depending on chronicity of the disease, hydration status and associated conditions, the fibrinogen concentration can be elevated and albumin concentration can vary. Hyperbilirubinemia is often observed and the liver enzyme activities like alkaline phosphatase (ALP), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) can be elevated because of reduced blood flow to the liver (Zobba *et al.,* 2008). Hypophosphatemia and hypoferremia are common, due to altered RBC metabolism (Frerichs and Holbrook, 1974). Ambawat *et al*. (1999) concluded that in this disease, a gradual decrease in Hb value was observed at various stages of parasitaemia and a sharp fall occurs when parasitaemia reached more than 50 per cent. Total serum bilirubin, urea, creatine kinase (CK) and lactate dehydrogenase (LDH) particularly in *T. equi* infected horses present increased levels (Camacho *et al.,* 2005).

In relation to clinical pathology, on gross examination the equids might demonstrate evidence of anemia as well as varying degrees of icterus, splenomegaly, edema, pulmonary edema, congestion, cardiac hemorrhages, hydro pericardium, hydrothorax, hepatomegaly, ascites, enlarged discolored kidneys and lymphadenopathy (de Waal, 1992). Pulmonary tissue examination can demonstrate oedema, congestion and hemosiderin-laden macrophages within the pulmonary alveolar walls. Histopathological findings are renal tubular necrosis with haemoglobin casts, centrilobular necrosis of the liver, necrosis of hepatocytes and micro thrombi within the liver and lungs (de Waal and van Heerden, 2004). Pronounced jaundice of serous membranes and pulmonary oedema are more prominent in *B. caballi* than in *T. equi* infections, whereas general lymphadenopathy has been observed in the latter (Phipps and Phipps, 1996).

Worldwide status of EP

The geographic distributions of *B. caballi* and *T. equi* are similar and include most of the world's tropical and subtropical regions (Brüning 1996). In most regions of the world where EP is endemic, *T. equi* infections are more prevalent than *B. caballi* infections (Rothschild and Knowles 2007). Both *B. caballi* and *T. equi* were detected in zebras from two national parks in South Africa by serological and culture methods (Zweygarth *et al* 2002). *B. caballi* and *T. equi* has not established in Australia or New Zealand (Rothschild and Knowles 2007). *B. caballi* infection has been reported in Southern and Eastern Europe, Asia, Africa, Middle East, Cuba, South and Central America as well as certain parts of the Southern United States (Ogunremi *et al* 2008). In

tropical and subtropical regions of Africa, Asia, countries deriving from the former Soviet Union and in all coastal countries of the Mediterranean the prevalence of *T. equi* is high. Thus it might be introduced into most countries worldwide (Mehlhorn and Schein 1998). In India first case of EP due to *T. equi* was reported from a stud farm at Hisar, Haryana due to import of exotic horse from Germany (Gautam and Dwivedi 1976). The prevalence of this parasite has been observed by different workers by microscopic examination, serological and molecular techniques. The recent details on prevalence of EP in equines by different methods as described by Onyiche *et al.* (2019). Brief from this are noted as under:

a. Blood smear examination-

Nigeria (83.3 and 11.1 for *B. caballi* and *T. equi,* respectively), Iran (0 and 5.0 of *B. caballi* and *T. equi,* respectively), Malaysia (22.2 and 16.9 of *B. caballi* and *T. equi,* respectively), Turkey (0 and 4.8 of *B. caballi* and *T. equi,* respectively)

b. Immunofluorescence antibody test (IFAT)-

Egypt (17.0 and 23.9 of *B. caballi* and *T. equi,* respectively), United Arab Emirates (10.5 and 33.3 of *B. caballi* and *T. equi,* respectively), Saudi Arabia (7.5 and 10.4 of *B. caballi* and *T. equi,* respectively), Iran (2.0 and 48.0 of *B. caballi* and *T. equi,* respectively), Thailand (11.1 and 3.2 of *B. caballi* and *T. equi,* respectively), Mexico (27.4 and 45.2 of *B. caballi* and *T. equi,* respectively), Switzerland (1.5 and 4.4 of *B. caballi* and *T. equi,* respectively), Netherlands (3 and 1 of *B. caballi* and *T. equi,* respectively), Hungry (31.8 of *T. equi*), Spain (21.0 and 44.0 of *B. caballi* and *T. equi,* respectively)

c. Enzyme-linked immunosorbent assay (ELISA)-

Egypt (14.8 and 0 of *B. caballi* and *T. equi,* respectively), Nigeria (4.4 and 65.6 of *B. caballi* and *T. equi,* respectively), United Arab Emirates (15.2 and 32.5 of *B. caballi* and *T. equi,* respectively), Korea (0 and 1.1 of *B. caballi* and *T. equi,* respectively), Jordan (0 and 14.6 of *B. caballi* and *T. equi,* respectively), Mongolia (51.6 and 19.6 of *B. caballi* and *T. equi,* respectively), China (51.2 and 11.5 of *B. caballi* and *T. equi,* respectively), Thailand (1.6 and 0 of *B. caballi* and *T. equi,* respectively), Malaysia (63.1 and 51.3 of *B. caballi* and *T. equi* respectively), Indonesia (0.4 and 1.7 of *B. caballi* and *T. equi,* respectively), Brazil (69.6 and 26.6 of *B. caballi* and *T. equi,* respectively), Venezuela (23.2 and 14.0 of *B. caballi* and *T. equi,* respectively), Costa Rica (69.2 and 88.5 of *B. caballi* and *T. equi,* respectively), Greece (1.1 and 9.2 of *B. caballi* and *T. equi,* respectively), Italy (8.9 and 39.8 of *B. caballi* and *T. equi,* respectively), Spain (6.5 and 53.7 of *B. caballi* and *T. equi,* respectively)

d. Compliment Fixation Test-

Brazil (54.6 and 28.5 of *B. caballi* and *T. equi,* respectively), France (12.9 and 58.0 of *B. caballi* and *T. equi,* respectively)

e. Polymerase chain reaction (PCR)-

South Africa (0 and 12.1 of *B. caballi* and *T. equi,* respectively), Tunisia (0.9 and 11.5 of *B. caballi* and *T. equi,* respectively), Egypt (19.3 and 36.4 of *B. caballi* and *T. equi,* respectively), Mongolia (42.4 and 6.4 of *B. caballi* and *T. equi,* respectively), Jordan (7.3 and 18.8 of *B. caballi* and *T. equi,* respectively), Korea (0 and 0.9 of *B. caballi* and *T. equi,* respectively), Thailand (0 and 0 of *B. caballi* and *T. equi,* respectively), Iran (0 and 45.0 of *B. caballi* and *T. equi,* respectively), Turkey (0 and 8.8 of *B. caballi* and *T. equi,* respectively), Indonesia (2.1 and 6.4 of *B. caballi* and *T. equi,* respectively), Costa Rica (20 and 46.2 of *B. caballi* and *T. equi,* respectively), Cuba (25 and 73 of *B. caballi* and *T. equi,* respectively), Venezuela (4.4 and 61.8 of *B. caballi* and *T. equi,* respectively), Brazil (60 and 38.5 of *B. caballi* and *T. equi,* respectively), Netherlands (1.6 of *T. equi*), Hungry (15.1 of *T. equi*), Romania (2.2 and 20.3 of *B. caballi* and *T. equi,* respectively), Italy (10.3 and 70.3 of *B. caballi* and *T. equi,* respectively)

Detail prevalence of EP in in other equids (Donkey/ mules etc.)-

a. Blood smear examination-

Ethiopia (1.8 and 12.2 of *B. caballi* and *T. equi,* respectively)

b. Complement-enzyme linked immuno sorbent assay (cELISA)-

Egypt (0 and 18.0 of *B. caballi* and *T. equi*, respectively), Kenya (0 and 81.2 of *B. caballi* and *T. equi,* respectively)

c. ELISA-

Thailand (3.4 and 7.3 of *B. caballi* and *T. equi,* respectively), Spain (32.1 and 66.1 of *B. caballi* and *T. equi,* respectively), Spain (17.0 and 47.2 of *B. caballi*

and *T. equi,* respectively), Brazil (73.9 of *T. equi*)

d. IFAT-

Ethiopia (13.2 and 55.7 of *B. caballi* and *T. equi,* respectively), Egypt (22.3 and 26.6 of *B. caballi* and *T. equi,* respectively), Thailand (2.8 and 10.7 of *B. caballi* and *T. equi,* respectively), Italy (40.6 and 47.80 of *B. caballi* and *T. equi,* respectively), Brazil (93.2 of *B. caballi*)

e. PCR-

Egypt (18 and 38.8 of *B. caballi* and *T. equi,* respectively), Thailand (0 and 1.7 of *B. caballi* and *T. equi,* respectively), Brazil (20.5 and 31.8 of *B. caballi* and *T. equi,* respectively), Italy (17.4 and 3.4 of *B. caballi* and *T. equi,* respectively)

By using molecular test like PCR *B. caballi* and *T. equi* has been also reported from dogs and camels in various country-.

Dog-

Spain, Portugal and France (40.0 of *T. equi*), Croatia (1.3 and 1.3 of *B. caballi* and *T. equi,* respectively), France (0.6 and 19.0 of *B. caballi* and *T. equi,* respectively), Paraguay (0.3 of *T. equi*)

Camels-

Jordan (60.0 and 40.0 of *B. caballi* and *T. equi,* respectively), Iraq (39.5 and 23.7 of *B. caballi* and *T. equi,* respectively)

Indian status of EP along with risk factor-In India very less amount of work has been conducted on EP. Brief details are as per Table1.

Risk factor associated with EP

Some important risk factor associated with EP are species, tick presence, age, sex, deworming status, other domesticated animal, management of farm, vaccination status etc. Donkeys/mules are more at risk with higher EP (*T. equi*) occurrence rate than horses (Rebiro *et al.,*1999; Rüegg *et al.,* 2007; Sumbria *et al.,* 2016a) this is due to fact that, donkeys/mules are kept mainly outdoor under deprived managemental livelihood state as they are mainly used for everyday

Table 1. State wise prevalence of equine piroplasmosis in India

S. no	State	Blood smear examination	cELISA	I-ELISA	Primary PCR	Nested PCR	IFAT	Capillary agglutination test
1	Punjab	3.66 (*T. equi*) (Sumbria *et al.,* 2017)	75 and 1.11 of *B. caballi* and *T. equi* respectively (Sumbria *et al.,* 2016a)	49.78 (*T. equi*) (Sumbria *et al.,* 2016b)	11.64 (*T. equi*) (Sumbria *et al.,* 2017)	21.77 (*T. equi*) (Sumbria *et al.,* 2016b)	58.33 (*T. equi*) (Sumbria *et al.,* 2018a)	-
2	Haryana	-	-	60.39 (*T. equi*) (Dahiya*et al.,* 2018)	-	-	-	38.3 (*T. equi*) (Malhotra *et al.,* 1978)
3	Rajasthan	-	-	71.40 (*T. equi*) (Dahiya*et al.,* 2018)	-	-	-	96.4 (*T. equi*) (Malhotra *et al.,* 1978)
4	Gujarat	-	-	48.92 (*T. equi*) (Dahiya*et al.,* 2018)	-	-	-	-
5	Uttar Pradesh	-	-	-	-	-	-	47.2 (*T. equi*) (Malhotra *et al.,* 1978)

Moreover *T. equi* has also been found in tick from Punjab and Himachal Pradesh (HP) region. In HP out of 74 tick 6.75% were positive for *T. equi* by PCR (Kashyap *et al.,* 2014). In Punjab out of 84 tick 31.15% were positive for *T. equi* by nested PCR (Sumbria *et al.,* 2018b).

transport and other farm activities, so as a result their chance of contact with ixodid ticks are more. At the same time thorough bred horses get balanced meals, proper veterinary care facility, so there is low chance of infection in horses (Steiman *et al.,* 2012). Equid on which more ixodid ticks exist, presented more rate of EP. In northern part of India *H. a. anatolicum* ticks spread *T. equi* (Bhagwan *et al.,* 2015) so more tick presence on equid mean more chance of infection. Both young and adult equids have same level of infection because farmer take care of both adult and foal in the similar manner. In relation to sex it was seen that female equids are more prone to EP as compared to male counterpart (Peckle *et al.,* 2013). This is may be due to the fact that mainly stallion is used for breeding purpose so strict living standard is used for stallion moreover more female are kept by farm owner because female has both draught and breeding potential (Shkap *et al.,*1998; Rebiro *et al.,*1999; Rüegg *et al.,*2007; Sigg *et al.,*2010).

Equids with proper deworming/vaccination schedule has less chance of infection as in India, equids are generally immunized against many diseases (equine influenza virus and tetanus), but they are not vaccinated against *T. equi* due to non-availability of any vaccine. So others infections together with neglected managemental practices can reduce the immunity level in non-vaccinated equids (Garcia-Bocanegra *et al.,*2013; Sumbria *et al.,*2016a). As it was seen that EP was also reported from dog and camels so due to lack of host specification by EP chance of infection in equids who share the living place with additional local domesticated animals is more (Garcia-Bocanegra*et al.,* 2013; Sumbria *et al.,*2017; Onyiche *et al.,* 2019). Due to better and advance managemental preparation and disease control programs the chance of EP is less in equid kept for recreational purposes as compared to the equids used for commercial purposes (Kouam *et al.,*2010; Sumbria *et al.,* 2016). Equids kept in unorganized stud farm has more chance of EP infection as in unorganised stud farm there is more chance of unhygienic open grazing, non-grooming practices moreover at the same time the chances of direct contact with ticks is also more in unorganised stud farm (Sumbria *et al.,*2017).

Diagnosis

Generally, the disease is diagnosed on the basis of clinical evidences augmented with some parasitological, serological tests or molecular tests. The clinical signs of piroplasmosis are variable and often non-specific (Bashiruddin *et al* 1999). *T. equi* associated intrauterine infection to the fetus is the most serious complication of the infection (Chhabra *et al* 2012) with deaths in rare hyperacute cases. Presumptive diagnosis can be made based various points including history of animal, history of vector, clinical sign in animal. Final diagnosis is made with the help of various classical parasitological and other advanced serological and molecular techniques.

Parasitological diagnosis:

On examination of Ramanowsky (Giemsa, Wright or Leishman) stained thin blood smear in side RBC *B.caballi* size reach up to 3-6 µm, on the other hand, size of *T. equi* inside RBC reach up to 1.5-2.5 µm. *Theileria equi* trophozoites are small erythrocytic stage appear as round, spindle or elliptical-shaped basophilic structures with merozoite stage appearing as two or four (Maltese cross) pyriform parasites, whereas on the other side the trophozoites of *B. caballi* appear as round, elliptical or oval basophilic structures. This organism commonly found in a single erythrocyte; appear mainly in pairs forming an acute angle (Edwards *et al* 2005). Slide examination is mainly useful in acute phase. Care should be taken to avoid false negative result, so slide should be examined by a skilled worker.

In-Vitro Culture- Micro aerophilus Stationary Phase (MASP):

MASP techniques is mainly used for *Babesia bovis* and *B. bigemina* culturing. This technique use defibrinated blood from infected animal, this blood is then suspended to final packed cell volume of 5-10% in a medium containing 40% FBS. This technique is carried out in low oxygen atmosphere and the culture once made can be maintained for 3 months (Vega *et al.,* 1985). Now a days MASP techniques is used for culturing of *T. equi* and *B. caballi*. The environmental condition for *in vitro* culturing of *T. equi* requires a humid gas mixture of 2% O_2, 5% CO_2 and 93% N_2 as compared with humidified 5% CO_2-in-air (Kumar and Kumar, 2007). *T. equi* can also be cultured at normal oxygen tension in the incubator in two special culturing media (SFRE-SFRE and HL-HL medium), (Zweygar*et al.,* 1997). For MASP culturing skilled hand are required moreover it is expensive technique and the positive confirmation of parasite require 2-10 days'post plating (Kumar and Kumar, 2007).

Serological techniques-

Compliment fixation test (CFT)

This test came into light in 1945 (Hirato *et al.,*1945). This test was first used in USA in 1970 and remain as official test till 2005. This test can't be used in animal like zebra/donkey because zebra/donkey has anti-complementary reaction. The test can give result at 8-day post infection but its sensitivity goes down after 2-3 months' post infection (Mcguire *et al.,* 1971)

Enzyme-linked immunosorbent assay (ELISA)

The test is highly sensitive and specific serological test (Kappmeyer *et al.,* 1999). This test can diagnose infection on 21 days to 5-week post infection. OIE declared cELISA as official test for screening of equids for international trade in 2004 (OIE 2005). Large number of antigen are used in EP, like Recombinant *T. equi* (EMA-1; EMA-2; Be82 and Be158) and *B. caballi* proteins (RAP-1; Bc48; Bc134) (Ristic and Sibinovlc, 1964).

Immunochromatographic test (ICT)

ICT is grounded on lateral/capillary flow technique. In ICT Abs or Ags are kept on strip of paper or nitrocellulose membrane. This test is not commercially available at field level, but has only been used in lab for research purpose (Onyiche *et al.,* 2019).

Indirect Immunofluorescent Antibody Test (IFAT)

This test first came into light in 1964 and is more sensitive than CFT (Ristic and Sibinovlc, 1964). It should be always used along with CFT. This test still remains as prescribed test by OIE. It can detect the infection on 3-20 days' post infection and can also be used in chronic case of infection. The main disadvantage of this test is that it is time consuming process and is difficult to standardised (Madden and Holbrook. 1968).

Molecular techniques

Molecular techniques mainly target DNA of parasite and amplify it. The most important molecular technique is polymerase chain reaction. PCR is highly sensitive and specific (Rampersad *et al.,* 2003). There are many variations of PCR like as conventional or primary PCR (one set of primers); real-time PCR (quantifies the level of parasite in peripheral blood); nested PCR (two sets of primers used to increase sensitivity) and nested PCR with hybridization (probe specific for gene target results in enhanced sensitivity and specificity). Nested PCR can detect *T. equi* up to a parasitemia of 0.000006% (Nicolaiewsky *et al.,* 2001). Other techniques which are attaining attention are reverse line blot hybridization and loop-mediated isothermal amplification (LAMP)

Treatment and prevention

Various drug has been tried to cure the cases of equine piroplasmosis. Bisazo dyes such as Trypan blue had been used against *B. caballi* in older days. In *in-vitro* test some compound are seen to be effective against EP like clotrimazole, ketoconazole, clodinafop-propargyl, artesunate, pyrimethamine, pamaquine, nitidine chloride, camptothecin, Lumefantrine and o-choline (Maji *et al*. 2019; Onyiche *et al.,* 2019). Many drugs including Pirevan, oxytetracycline, diminazene and buparvaquone have been used with variable success to treat babesiosis in horses. In India some studies on drug efficacy testing against experimental infection of *T. equi* has been furnished (Kumar *et al* 2003). Little emphasis has been given on testing the efficacy of drugs effective against natural infection of EP.

Being a serious threat to the productivity of equine population the condition of EP is to be treated successfully so as to eliminate the parasite and make animal free of carrier state. In endemic regions, treatment of piroplasmosis is used only as a means of decreasing clinical signs and reducing fatalities. Clearance of the organism serves no purpose in these countries as life-long immunity (premunity) is assumed to be conferred with chronic, inapparent infection. In non-endemic regions attempting to remain free of piroplasmosis, treatment of infected horses with the intent of clearance (chemosterilization) is desired. *T. equi* infections are more typically difficult to treat than *B. caballi* infections. As discussed above, numerous drugs have been reported to have variable efficacy in inhibiting *T. equi* and *B. caballi* both in cell culture and *in-vivo*. Prevention of EP is very important to control the spread of infection. Through inspection of equids should be done for the presence of ticks, tick infected equids should be separated from rest of equids, to control the tick various acaricides along with biological control should be used (Sumbria and Singla, 2015).

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